DNA-BASED MALARIA VACCINES

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MIDRP on the Frontiers of Modern (Molecular) Biomedicine

Malaria as the DoD's single most important infectious diseases threat

Malaria as a model system for developing the technology and insights to *rapidly develop vaccines against numerous infectious diseases and BW threats*

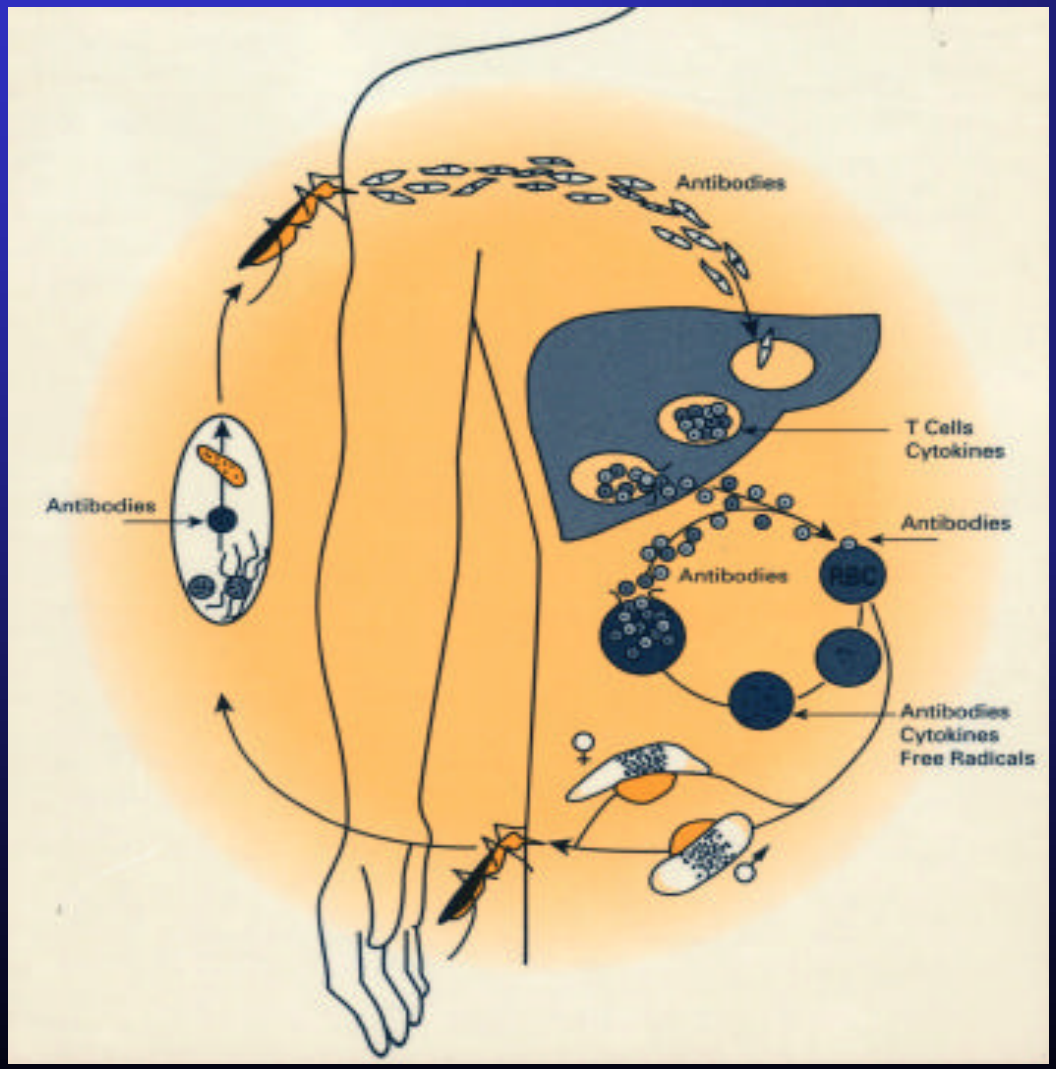
Warfighter Protection

	New cases of Malaria	Sick days lost
WWII	605,555	12,000,000
Vietnam	65,020	1,186,465

Pacific Theatre during WWII: Attack rates exceeded 2 cases / Marine / year in some battalions.

Somalia: 500 Marines stationed in Baardera for 1 month, on prophylaxis. 53 malaria cases (attack rate of 10.6% per month); transmission intensity in Baardera is 1/10th that of other areas of Africa.

PLASMODIUM - ETIOLOGIC AGENT OF MALARIA



- Multistage life cycle with stage-specific expression of proteins.
- Large genome: 26-30 mega-bases, 5-6000 genes, on 14 chromosomes.
- Allellic and antigenic variation.
- Complex, genetically variable, human immune response.

A MALARIA VACCINE IS FEASIBLE

PRE-ERYTH STAGE: IRRADIATED SPOROZOITE MODEL

- Greater than 95% protection, not strain specific, lasts for at least 9 months.
- CD8+ T cells target parasite proteins expressed on surface of liver cells, antibodies target sporozoites.
- Vaccine not amenable to licensure.

BLOOD STAGE: NATURALLY ACQUIRED IMMUNITY

- Protects against disease and death in life-long residents of malaria endemic areas.
- Antibodies target merozoites and infected erythrocytes.

Proposed TWO-TIERED Vaccine

PRE-ERYTHROCYTIC STAGE VACCINE:

Prevent all clinical manifestations of malaria in 95% of vaccine recipients for at least six months.

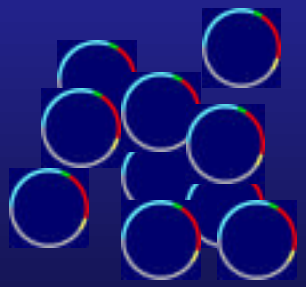
BLOOD STAGE VACCINE:

Prevent severe disease and death in the 5% break-through infections (2nd line of defense).

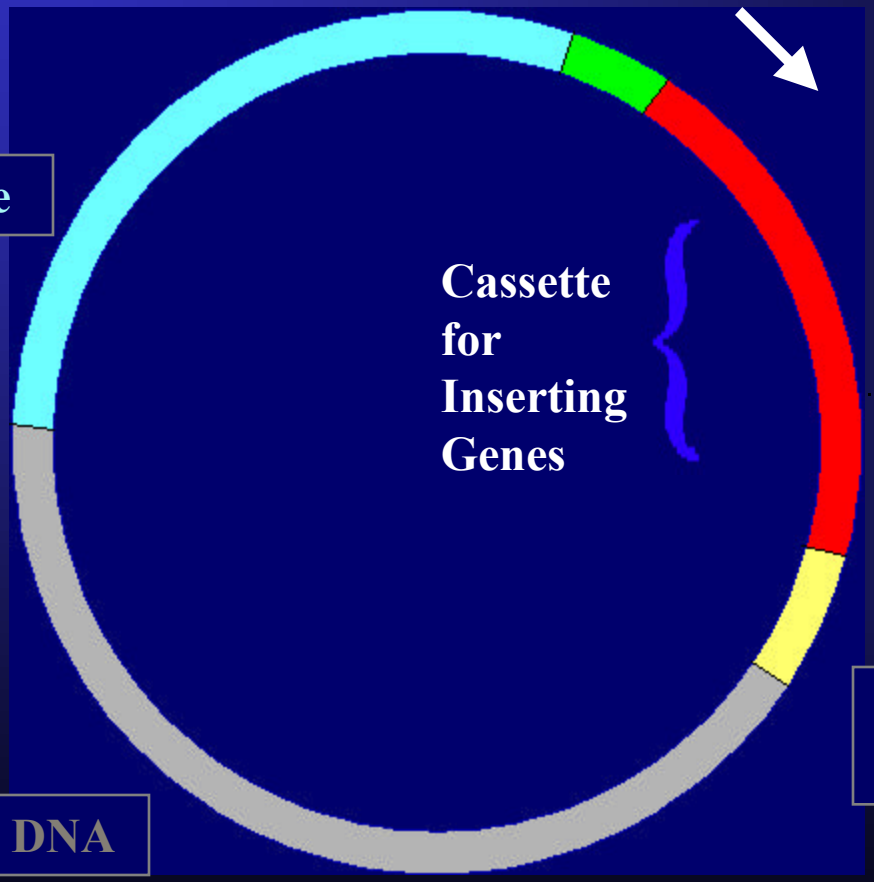
DNA VACCINES ARE THE ONLY AVAILABLE TECHNOLOGY FOR CREATING SUCH A MULTI-STAGE, MULTI-VALENT, MULTI-IMMUNE RESPONSE VACCINE

DNA Vaccine Design

Ring of dsDNA



The vaccine



New Platform Technology

- **DNA vaccines are fundamentally different**

  Instead of administering foreign material, human cells produce the foreign material using the DNA as a template.

- **Advantages of DNA vaccines**
  - **Easy/relatively inexpensive to produce, purify.**
  - **May not require a cold chain.**
  - **Can be combined (multivalency) and modified. Highly immunogenic, especially for CD8+ T cells.**

⇨ Provides potential for RAPID production of new vaccines.

1st CLINICAL TRIALS OF MALARIA DNA VACCINE -- 1 Gene

- Principals: NMRC, Vical, Aventis-Pasteur
- Location: USAMRIID.
- 35 Volunteers received 1- gene vaccine.
- Results:
  - Safe and well tolerated.
  - Excellent induction of CD8+ CTL.
  - Historic result.

Cutting Edge Research

Induction of Antigen-Specific Cytotoxic T Lymphocytes in Humans by a Malaria DNA Vaccine

Ruobing Wang,* Denise L. Doolan,* Thong P. Le,†
Richard C. Hedstrom, Kevin M. Coonan, Yupin Charoenvit,
Trevor R. Jones, Peter Hobart, Michal Margalith, Jennifer Ng,
Walter R. Weiss, Martha Sedegah, Charles de Taisne,
Jon A. Norman, Stephen L. Hoffman‡

$CD8^+$ cytotoxic T lymphocytes (CTLs) are critical for protection against intracellular pathogens but often have been difficult to induce by subunit vaccines in animals. DNA vaccines elicit protective $CD8^+$ T cell responses. Malaria-naive volunteers who were vaccinated with plasmid DNA encoding a malaria protein developed antigen-specific, genetically restricted, $CD8^+$ T cell–dependent CTLs. Responses were directed against all 10 peptides tested and were restricted by six human lymphocyte antigen (HLA) class I alleles. This first demonstration in healthy naïve humans of the induction of $CD8^+$ CTLs by DNA vaccines, including CTLs that were restricted by multiple HLA alleles in the same individual, provides a foundation for further human testing of this potentially revolutionary vaccine technology.

Science
October 1998

"This first demonstration in healthy naïve humans ... provides a foundation for further human testing of this potentially revolutionary vaccine technology."

1st Clinical Trial of 5-Gene Vaccine

- First injection on August 22nd.
- Volunteers will be challenged with malaria.
- We do not expect good protection with this first generation vaccine. Improvements:
  - Incorporation of GM-CSF cytokine.
  - Incorporation of prime boost vaccination.
- Second generation in GMP production.
  - Synthetic genes.

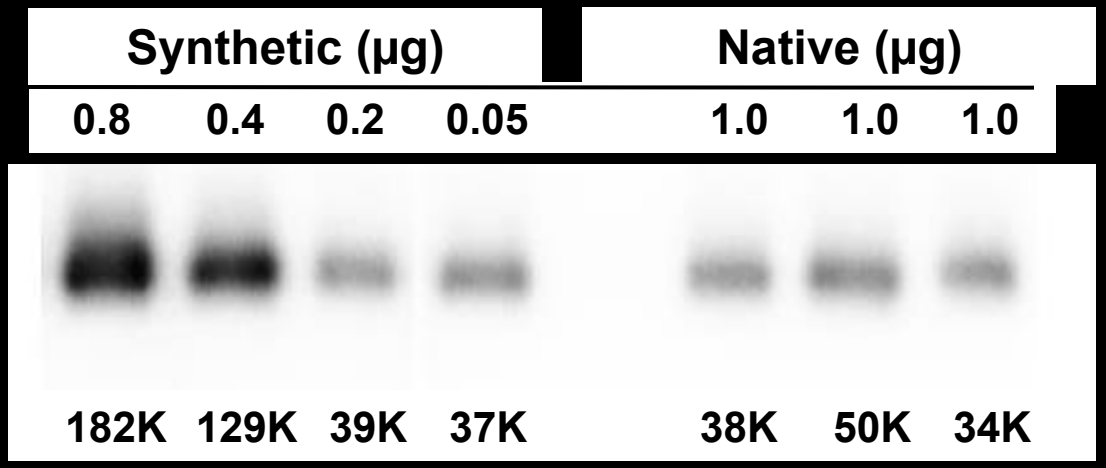
Adding GM-CSF expressing plasmid to prime increases protection, IFNγ

Prime-Boost	Prot/Chal	Protection	IFNγ-ELIspot
DG-V	8/10	80%	3440 (/10^6 cells)
D-V	5/10	50%	900
DG-DG	3/10	30%	580
D-D	1/10	10%	360
dg-V	6/10	60%	1460
0.1 dg-V	3/10	30%	840

2 immuniz's, 3 wks apart: D=100, d=1; G=30, g=1 μg.

Sedegah, Weiss et al, J Immunol, June 2000

CHANGING CODON USAGE INCREASES EBA-175 PROTEIN EXPRESSION

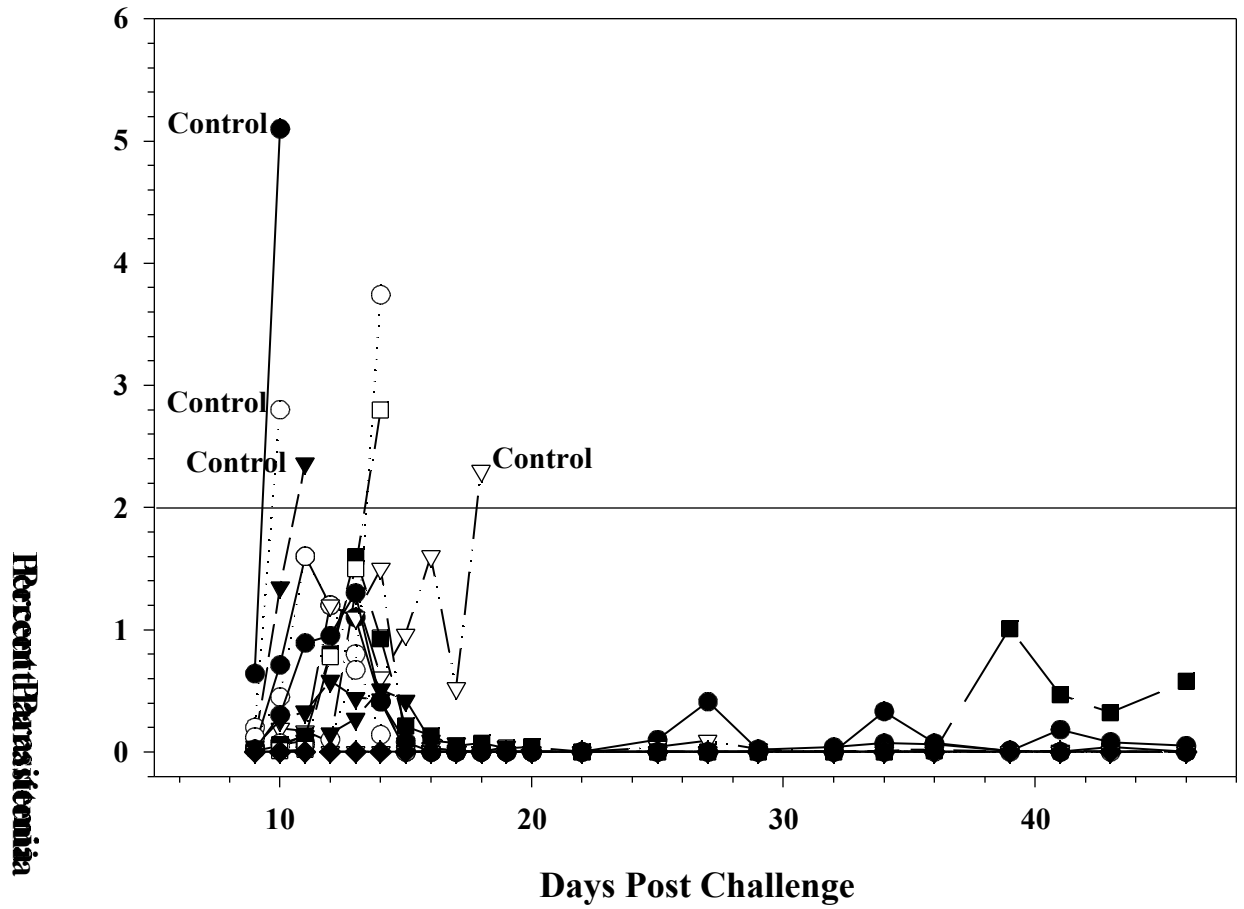


1st 2-Tiered Vaccine in Primates

- Rigorous Animal Model: *P. knowlesi* in Rhesus Monkeys.
- Immunized with 4 gene DNA vaccine.
  - Two liver stage antigens.
  - Two blood stage antigens.
- Combined with cytokine genes.
- Boosted with recombinant poxvirus.

→ *4/4 control monkeys needed treatment, vs. 2/11 vaccinated monkeys*

Parasitemias in Challenged Monkeys



Combined Vaccine for Humans

- We are now progressing to an EIGHT GENE combined pre-erythrocytic (5 genes) / erythrocytic (3 genes) vaccine incorporating OPTIMIZED CODON USAGE and amenable to boosting with proteins and/or poxviruses.

Professionalism in Product Development

- FDA oversight, ethical review.
- Corporate collaborators
  - Biotech (Vical, Chiron, Entremed).
  - Big Pharma (Aventis Pasteur, SB Bio).
- Formal Advisory Boards of Leading Scientists.
  - Vaccinology (Wyeth Lederle), Field Trials, and Parasitology.

SUMMARY

- Malaria is DoD's most important ID threat, 100 yrs.
- Extremely difficult and complex problem, but is an important model for many other problems.
- NMRC is one of the world's leaders in (malaria) vaccinology and functional genomics.
- DNA-based vaccines are a revolutionary technology with the promise of creating "real time" vaccines for multiple infectious diseases of military importance and against the agents of biological warfare.